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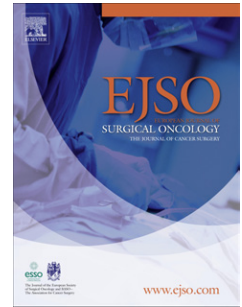
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VEGF_t / VEGF_{nt} ratio as a good marker for survival in colorectal cancer patients?

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.Abbreviations: VEGF: vascular endothelial growth factor. VEGF_t: VEGF performed in tumour sample VEGF_{nt}: VEGF performed in non-tumour sample ELISA: enzyme-linked immunosorbent assay.

Abstract

Aims: Vascular endothelial growth factor (VEGF) over expression is frequently considered as a marker of poor prognosis and of an aggressive tumour phenotype. Colorectal carcinoma is still one of the most lethal malignancies. Thus, our purpose was to study the expression of VEGF in tumour tissue and around these tumours and analyze its correlation with clinico-pathological features and overall-survival.

Methods: The study was designed to determine the concentration of vascular endothelial growth factor in tumour (n=87) and non-tumour tissue (n=230) obtained from the colorectal cancer patients. Accordingly, VEGF expression was studied in tissue homogenates by a quantitative sandwich ELISA method.

Results: The study was performed on 317 colorectal samples from 87 colorectal cancer patients. VEGF expression was higher in the tumour than in the non-tumour area ($P < 0.0005$). In areas 5-10 cm around the tumours, VEGF expression were higher than obtained in proximal or distal edge of the resection. VEGF_t expression was lower in patients with stage I than in patients with stage II, III, or IV. However, a shorter overall survival time was evident when the ratio obtained between VEGF expression in the tumour and mean VEGF expression in the non-tumour areas of the same patient (VEGF_t/VEGF_{nt} ratio) was ≤ 2 ($P = 0.019$).

Conclusions: VEGF expression in colorectal cancer tissue was higher in tumour than in non-tumour areas. VEGF_t expression was lower in initial clinical stages. Indeed, patients who presented a VEGF_t/VEGF_{nt} ratio > 2 survived longer. This is the first report showing an association between VEGF_{nt} over-expression and clinical outcome.

Keywords: VEGF; colorectal cancer; overall-survival; clinico-pathologic features; immunoassay.

Introduction

Angiogenesis is essential in several physiological processes, for example in the development of embryonic tissue and in placental formation. As such, the induction of angiogenesis is regulated through the expression of different angiogenic and anti-angiogenic factors. Indeed, it is well known that angiogenesis is primordial for solid tumour growth and dissemination.¹ Binding of VEGF and other ligands to the VEGF-receptor (VEGFR) of endothelial cells activates the angiogenic pathway. Accordingly, the role of vascular endothelial growth factor (VEGF) in tumour angiogenesis has been studied extensively.

VEGF is strongly expressed in many human cancers, including oesophageal carcinomas^{2, 3} as well as in intestinal⁴ and breast cancers.⁵⁻⁷ This growth factor is correlated with disease progression and lower overall survival. Serum VEGF levels are elevated in colorectal cancer patients.⁸ Indeed in hepatocellular carcinoma, it is considered marker of poor prognosis.^{9, 10}

New therapeutic approaches targeting VEGFR have been developed and anti-VEGFR blocking antibodies are being used in distinct clinical trials (phase I and II) aimed at treating different malignant diseases. Such specific anti-angiogenic therapies do not intend to eliminate tumour cells but rather, they focus on disease stabilization.¹¹⁻¹³ Anyway some authors considered that addition of bevacizumab (a monoclonal antibody against VEGF) plus chemotherapy may improve overall survival rate in colorectal cancer patients.¹⁴

The aim of the present study was to assess the prognostic value of VEGF expression in colorectal tissue when compared to other prognostic clinical parameters (age, gender, clinical stage, lymph node involvement, etc.), and to determine its relationship with clinical outcome and overall survival in colorectal cancer patients.

Patients and methods

Patients

The patients were diagnosed and treated in the Department of Surgery at the Hospital Universitario 12 de Octubre (Madrid, Spain). Patients who underwent colorectal cancer resection operations (1997-1999) were reviewed between September 1997 and July 2009. The diagnosis and staging of colorectal cancer was performed according the WHO classification¹⁵ and the patient's stage was determined according to the International Union Against Cancer: TNM classification of malignant tumours.¹⁶ These categories were used to classify the patients: corresponds to stage I ($T_{1-2} N_0 M_0$), stage II ($T_{3-4} N_0 M_0$), stage III ($T_{any} N_{1-2} M_0$) and stage IV ($T_{any} N_{any} M_1$).

Written informed consent to analyse tumour markers was obtained from the patients before surgery and the study was approved by the Institutional Research Board (Ethical Committee) at the Hospital Universitario 12 de Octubre.

Freshly resected colorectal samples were obtained from 87 patients who consecutively underwent surgery for colorectal cancer. Each sample size was 1 cm³ approximately. Half of the samples were used for pathology diagnosis and the other macroscopically equivalent half was sent to laboratory. The samples

were immediately frozen in liquid nitrogen (N₂) and stored at -80°C until they were assayed.

The tissue samples were assigned to two different groups, one corresponding to colorectal tumours (t) and the other corresponding to non-tumour (nt) areas of each patient. The study was carried out on 317 colorectal tissue samples, corresponding to colorectal tumours (n=87) and 3 tissue samples corresponding to the non-tumour areas in each patient: proximal or oral edge (PE), n=69; from tissues 5 -10 cm away from the tumour but no tumoral (N), n=87; and distal or anal edge (DE), n=74. All tissue samples were examined histologically to confirm the diagnosis of adenocarcinoma and the presence or absence of tumour cells in non-tumour areas.

The clinical data, including the results of the follow-up were analyzed. All patients underwent clinical and biochemical evaluation, chest X-rays, colonoscopies (endoscopies) and computed tomography scans every 3-6 months to detect any recurrence.

Exclusion criteria established in our protocol: 1st some samples of non-tumour tissue were rejected as carcinoma cells were identified histologically. The second exclusion criterion was for survival analysis: patients who were followed - up less than 3 months.

VEGF concentrations by ELISA assay

VEGF concentrations were measured by ELISA (Quantikine human VEGF immunoassay, R&D Systems, Madrid, Spain) in tissue homogenates (supernatants) obtained as described previously.¹⁷ Optical densities (O.D.) were determined by the absorbance at 450 in an ELISA reader using Multiskan EX (Thermo Electron Corporation, Lab Center S.L., Madrid, Spain). The best-fit line

was determined by regression analysis and all the samples were assayed in duplicate. According to the method used, the minimum detectable dose was less than 9.0 pg / ml. To evaluate intra-assay variations in that 3 different tissue samples were assayed in 8 replicates at the same time. For inter-assay variations 3 tumour samples and 3 non-tumours were performed in duplicate for 2 consecutive days. The coefficient variation (CV) was minor than 10% respectively.

The median VEGF levels in the tissue homogenates were expressed as pg/mg of total protein and the protein concentrations were determined in the corresponding supernatant using the DC protein assay kit (Bio-Rad, Barcelona, Spain).

All values shown are the mean \pm standard error of media (SEM) performed in duplicate.

Statistical analysis

The Student's t test and Fisher exact tests were used to compare the proportions and the continuous variables were assessed with the Mann-Whitney *U* test. The primary statistical endpoint in this study was the overall survival measured from the date of surgery, and survival was assessed according to the Kaplan-Meier method (Graph Pad Prism 5). Statistical differences in survival were determined using the log-rank test. . All data were analyzed with SPSS.11.01 software for Windows (SPSS, Inc., Chicago, IL, USA) and a *P* value less than 0.05 was considered statistically significant.

In this study we considered as a cut-off for analysis of age, 69 years old. This age was calculated as the mean value of the 87 colorectal cancer patients old age.

Results

Clinical and pathological findings

Of the patients studied here 47 were male (47/87; 54.0%) and 40 were female (40/87; 46.0%). When grouped according to their age, 42 patients (48.3 %) were \leq 69 years old and 45 patients (51.7%) were older than 69 years of age. The site of the primary tumour was in the right colon in 18 cases (20.7%), in the left and sigmoid colon in 27 cases (31.0%), and in the rectum in 42 patients (48.3%). Of the colorectal cancer patients, 59 (67.8%) did not show lymph node involvement whereas in 28 patients the lymph nodes were affected (32.2%). According to the clinical stages, 7 patients were stage I (8.1%), 52 patients were stage II (59.8%), 23 patients were stage III (26.4%) and 5 patients were stage IV (5.7%).(Table 1)

At the time of censoring the data for analysis, 54 (62.1%) of the 87 patients had died, with a mean survival time of 72.5 months (range: 6-139 months). Of the patients that remained alive at the conclusion of the study n=33; (37.9%), the mean overall survival time was 86.5 months (range: 12–161 months).

VEGF expression in tissue homogenates

The mean VEGF_t expression in the male colorectal cancer patients was 292.3 ± 35.4 pg/mg protein (n=47) and in the female patients the VEGF_t expression was 218.5 ± 49.5 pg/mg protein (n=40). The VEGF_t concentration in patients \leq 69

years old (310.4 ± 36.0 pg/mg protein, $n=42$) was similar than that in patients > 69 years old (272.7 ± 46.3 pg/mg protein, $n=45$). In positive lymph node group shows VEGF_t with 296.3 ± 49.2 pg/mg protein, $n=28$ and in negative group was 283.6 ± 37.0 pg/mg, $n= 59$, (Table 1).

When the relationship between the VEGF_t and VEGF_{nt} was assessed, the differences obtained were highly significant. In the tumour areas, the VEGF_t concentration (289.2 ± 29.6 pg/mg protein, $n= 87$) was higher than in the non-tumour (VEGF_{nt}) areas (74.5 ± 8.3 pg/mg protein, $n= 215$), (Table 1).

VEGF_{nt} was analyzed according to the surgical resection situation in the colon. Thus, at the PE of the colon VEGF_{nt} expression was 61.2 ± 8.4 pg/mg of protein ($n= 69$), whereas in the N domain the VEGF_{nt} was 91.5 ± 12.9 pg/mg protein $n= 87$, (PE vs. N: $P < 0.025$) and at DE, the VEGF_{nt} expression was 67.2 ± 9.8 pg/ mg of protein $n=74$, (N vs. DE: $P < 0.10$; PE vs. DE: $P=0.35$). (Fig.1)

The mean expression of VEGF_t varied according to the clinical stage: in stage I, was 148.5 ± 16.8 pg/mg of protein ($n=7$); in stage II was 306.5 ± 40.9 pg/mg of protein ($n=52$); stage III, was 277.5 ± 48.9 pg/mg of protein ($n= 23$); and in stage IV was 488.7 ± 116.2 pg/mg of protein ($n=5$), Table 1.

In terms of the location of the tumour in the colon, the VEGF_t concentration in the Right colon (R) was 375.7 ± 98.0 pg/mg of protein ($n= 18$), in the Left and Sigmoid (L+S) colon it was 271.1 ± 44.2 pg/mg of protein ($n= 27$), and in the Rectum (Re) it was 268.6 ± 34.4 pg/mg of protein ($n= 42$); in R vs. L+S was $P < 0.20$; in L+S vs. Re was $P < 0.47$; and in R vs. Re was $P < 0.15$.

In this study the cut-off level of ratio (VEGF_t/VEGF_{nt}) was determined. This level was determined between the VEGF expression in the tumour (VEGF_t) and in the non-tumour tissue (VEGF_{nt}). The mean of three VEGF expression

values in non-tumour areas (PE+N+DE/3) were taken as the denominator for ratio calculation in each patient. The patients were considered on the basis of the different $VEGF_t/VEGF_{nt}$ ratios and the survival was analyzed by Kaplan-Meier method. Two groups ($VEGF_t/VEGF_{nt} \leq 2$ and $VEGF_t/VEGF_{nt} > 2$) were established by applying a cut-off level of 2, where the survival data was statistically significant.

Comparison of survival curves: Overall survival of colorectal cancer patients in different stages

The overall survival time of colorectal cancer patients was analyzed and the patients were grouped according to the different clinical stages (without considering the VEGF values). Grouped as stage I+II (median survival was 114 months) versus stage III+IV (median survival was 84 months), was (hazard ratios) OR 0.6921, 95% CI 0.3474 -1.292; $P= 0.23$. A statistical analyses according Kaplan-Meier method failed to find significant differences between these groups.

Overall survival of colorectal cancer patients with $VEGF_t/VEGF_{nt}$ ratio

Patients with a $VEGF_t/VEGF_{nt}$ ratio > 2 ($n= 23$) survived longer (median survival time was 133.0 months) than patients with a $VEGF_t/VEGF_{nt}$ ratio ≤ 2 , $n= 57$; (median survival time was 62.0 months), was OR 2.024, 95% CI 1.144 – 4.629; $P< 0.0194$ (Fig. 2). There were 7 patients who were followed up for less than 3 months and they were excluded from the analysis.

Discussion

VEGF fulfils an important role in tumour induction and progression, and administration of monoclonal antibodies against VEGF decreases blood vessel density and suppress the growth of experimental tumours.¹⁸ VEGF has been evaluated in pathological specimens of colon carcinoma although generally by staining neoplastic cells by immunohistochemistry. Accordingly, an association has been found between VEGF overexpression and poor responsiveness to radiotherapy in rectal cancer.¹⁹ Also, the median survival time was shorter in patients with high VEGF expression than in those whom no VEGF staining was detected.²⁰ VEGF was considered to be an independent factor that affects survival or that can predict survival. In serum or plasma, VEGF over-expression was also considered as a marker to predict recurrence in colorectal cancer patients or associated with distant metastases.^{21, 22} Whereas experimentally VEGF serum expression does not appear to have diagnostic value, it could be of prognostic value in colorectal cancer patients.²³

To the best of our knowledge, there is no data regarding VEGF expression in tissue homogenates from colorectal carcinoma patients. Here we used a quantitative method to assess VEGF protein expression in tumour and non-tumour homogenates from colorectal cancer patients by the ELISA method, as has been performed previously with some other biomarkers in different tissues.^{17, 24, 25}

Zafirelli et al.²⁶ has been assessed VEGF expression by immunohistochemistry in tissue microarrays and the samples quantified by semi-automated computerized image analysis, indicating that VEGF expression was an independent prognostic marker for Stage III tumours.

We examined VEGF expression in colorectal tissue (tumours and non-tumours) corresponding to the each patients, to determine whether there is an association between VEGF over-expression and clinical established prognostic factors of the disease. Our results showed that, the mean VEGF_t expression was similar in younger patients when compared with patients older than 69 years of age. Also, when the tumour was located to the right area (R), the mean VEGF_t expression tends to be higher than in the rectum (Re). However, these results could be influenced by the levels in normal vascular areas or by inflammation process in the micro-environment around tumours. To eliminate possible unspecific binding, VEGF concentrations were normalized in each samples assessed, to the total protein concentration obtained.

Unexpectedly, and in contrast to other studies ²⁶ the mean VEGF_t expression was similar in patients with positive lymph node involvement than in those with no metastasis in the lymph node. Nevertheless, there were significant differences in the mean VEGF_t expression in terms of the different (TNM) stages when compared stage I versus stage II, III or IV. These results indicated that, VEGF expression only in tumour area could be used as a poor prognostic parameter.

In our study median VEGF expression in tumour areas was higher than in non-tumour areas. However, when VEGF expression was analysed in each patients individually, in many cases, VEGF expression in non-tumour areas was higher than in corresponding tumour areas. This allows us to analyse the ratio of VEGF expression between tumour and non tumour areas, (VEGF_t/VEGF_{nt}). Indeed, when the patients were considered on the basis of the different VEGF_t/VEGF_{nt} ratios, two groups were established by applying a cut-off level of

2, where the survival analysis by Kaplan-Meier method was statistically significant.

When the overall survival time of our group of patients was studied according to the clinical stages, without considering the VEGF values, we did not find statistically significant differences in the results.

However, when the overall survival time of these patients was studied considering the $VEGF_t/VEGF_{nt}$ ratio, we found that patients with a higher $VEGF_{nt}$ expression in colorectal tissue than in $VEGF_t$, might have a poor prognosis ($VEGF_t/VEGF_{nt} \leq 2$) when compared to those with lower $VEGF_{nt}$ expression.

It is most likely that VEGF content may be modulated distinctly in tumour and in non-tumour tissue in colorectal cancer patients and that its expression may be altered as a consequence of neoplastic cell proliferation. Moreover, these results could indicate that VEGF represent target acting in the tumour microenvironment inducing the local dissemination of the tumour. Further studies are needed to elucidate if this patients with a $VEGF_t/VEGF_{nt}$ ratio ≤ 2 have shorter relapse times and therefore, need more frequent follow-up, and whether they might specially benefit from anti-angiogenic treatment^{27, 28} or to consider some associated therapy or even more aggressive treatments.

In our study when VEGF expression is not considered, there were not significant differences in the survival time among the colorectal cancer patients at different stages of the disease. This probable is related to the study size or due to the other factors that could affect the survival like age, other associated pathology, response to different therapies, etc. However, these same factors are affecting the survival study when evaluated according the $VEGF_t/VEGF_{nt}$ ratio and in this case we can observe significant differences in survival time and

find significant differences in survival time and, these ratio could be use as a valuable outcome factor.

Conclusions

In conclusion, our results show that mean VEGF expression in colorectal tumours was higher than in non-tumour areas and, have to point out when in colorectal cancer patients the VEGF values were lower in its non-tumour areas than in tumour areas, these patients survived longer, indicating that $VEGF_t/VEGF_{nt}$ ratio could be a valuable prognostic parameter for these patients.

Conflict of interest disclosures

The authors made no disclosures

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References

1. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990;82:4-6.
2. Bedoya F, Meneu JC, Macías MI, *et al.* Mutation in CNR1 gene and VEGF expression in esophageal cancer E. *Tumori* 2009;95:68-75.
3. Inoue K, Ozeki Y, Suganuma Y, Sugiura Y, Tanaka S. Vascular endothelial growth factor expression in primary esophageal squamous cell carcinoma: association with angiogenesis and tumor progression. *Cancer* 1997;79:206-13.
4. Bendardaf R, Buhmeida A, Hilska M, *et al.* VEGF-1 expression in colorectal cancer is associated with disease localization, stage, and long-term disease-specific survival. *Anticancer Res* 2008;28:3865-3870.
5. Li YJ, Deng YJ, Wen G, Zhang XL. Evaluation of angiogenesis in the tumorigenesis and progression of breast cancer. *Zhonghua Wai Ke Za Zhi* 2009;47:519-22.
6. Bluff JE, Menakuru SR, Cross SS, *et al.* Angiogenesis is associated with the onset of hyperplasia in human ductal breast disease. *Br J Cancer* 2009 101: 666-72.
7. Gisterek I, Matkowi R, Lacko A, *et al.* Serum vascular Endothelial growth factors A, C, and D in human breast tumors. *Pathol Oncol Res* 2009 DOI.10.1007/s12253-009-9211-18.
8. Mysliwiec P, Pawlak K, Kuklinski A, Kedra B. Combined preoperative plasma endoglobin and VEGF-A assessment on colorectal cancer patients. *Folia Histochem Cytobiol* 2008;46:487-92
9. Tang ZY, Ye SL, Liu YK, *et al.* A decade's studies on metastasis of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004;130:187-96.
10. Guo RP, Zhong C, Shi M, *et al.* Clinical value of apoptosis and angiogenesis factors in estimating the prognosis of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2006;132:547-55.

11. Wedge SR, Ogilvie DJ, Dukes M, *et al.* ZD4190: an orally active inhibitor of vascular endothelial growth factor signaling with board-spectrum antitumor efficacy. *Cancer Res* 2000;60:970-5.
12. Heckman CA, Holopainen T, Wirzenius M, *et al.* The tyrosine kinase inhibitor cediranib blocks ligand-induced vascular endothelial growth factor receptor-3 activity and lymphangiogenesis. *Cancer Res.* 2008;68:4754-62.
13. Goodlad RA, Ryan AJ, Wedge SR, *et al.* Inhibiting vascular endothelial growth factor receptor-2 signalling reduces tumor burden in the Apc Min/+ mouse model of early intestinal cancer. *Carcinogenesis* 2006;27:2133-9.
14. Hurwitz H, Fehrenbacher L, Novotny W, *et al* Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335-42.
15. World Health Organization. International histological classification of tumours, 2nd ed. Geneva, World Health Organization/ Berlin Springer-Verlag 1988-Present
16. Sobin LH, Wittekind Ch, eds. UICC "TNM Classification of Malignant Tumors", 6th ed., New York, Wiley. 2002
17. Morales-Gutiérrez C, Vegh I, Colina F, *et al.* Survival of patients with colorectal carcinoma. *Cancer* 1999;86:1675-81.
18. Kim KJ, Li B, Winer J, *et al.* Inhibition of vascular endothelial growth factor induced angiogenesis suppresses tumor growth in vivo. *Nature* 1993;362:841-4.
19. Inti Z, Steele R Compton CC. VEGF as a predictive marker of rectal tumor response to preoperative radiotherapy. *Cancer* 2005;104:2517-21.
20. Doger FK, Meteoglu I, Tunyurek P, Okyay P, Cevikel H. Does the EGFR and VEGF expression predict the prognosis in colon cancer? *Eur Surgical Res* 2006;38:540-4.

21. Alabi AA, Suppiah A, Madden LA, Monson JR, Greenman J. Preoperative serum vascular endothelial growth factor-a is a marker for subsequent recurrence in colorectal cancer patients. *Dis Colon Rectum* 2009;52:993-9.
22. Alabi AA, Suppiah A, Madden LA, Monson JR, Greenman J. Preoperative serum levels of serum VEGF-C is associated with distant metastasis in colorectal cancer patients. *Int J Colorectal Dis* 2009;24:269-74.
23. Gonzalez FJ, Quesada AR, Sevilla I, et al. Prognostic value of serum angiogenic activity in colorectal cancer patients. *J Cell Mol Med* 2007;11:120-8.
24. Vegh I, De la Calle Santiuste A, Colina F, et al. Relationship between biomarker expression and allelic alteration in esophageal carcinoma. *J Gastroenterol Hepathol* 2007;22: 2303-9.
25. Vegh I, De-la Cruz J, Navarro S, et al. Colorectal cancer relapse: Allelic alterations associated with tumor marker overexpression. *Oncology*. 2003;65:146-51.
26. Zafirellis K, Agrogiannis G, Zachaki A, Gravani K, Karameris A, Kombouras C. Prognostic significance of VEGF expression evaluated by quantitative immunohistochemical analysis in colorectal cancer. *J Surg Res* 2008;147:99-107.
27. Tol J, Koopman M, Cats A, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med* 2009;360:563-72.
28. Jonker DJ, O'Callahan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007; 357:2040-8.

Figure legends

Figure 1. - The mean VEGF expression in different colorectal tissues. Left: The mean $VEGF_t$ expression in the tumour versus the mean $VEGF_{nt}$ expression in non-tumour tissue was $P < 0.0005$. Right: The mean $VEGF_{nt}$ expression in the different non- tumour areas: PE= proximal or oral edge; N= 5 -10 cm. peritumoral area and DE= distal or anal edge. PE vs. N, $P=0.025$. N vs. DE: $P=0.10$ and PE vs. DE: $P=0.40$. NT indicate non-tumour tissue; T, tumour tissue (compared by Student's t test)

Figure 2. - Kaplan-Meier survival curves (overall survival) with patients with colorectal cancer. The survival of patients with the $VEGF_t/VEGF_{nt}$ ratio ≤ 2 was significantly worse than in patients with a ratio > 2 (compared by log-rank-test, $P= 0.019$). Survival was determined according to the Kaplan-Meier method.

Table 1.
Relationship between VEGF expression and clinicopathologic findings

Patients Characteristics	Total	VEGF pg/mg ±SEM	P value
Gender ^a			
Male	47	292.3 ± 35.4	0.49
Female	40	281.5 ± 49.5	
Age (years old) ^a			
≤ 69	42	310.4 ± 36.0	0.25
> 69	45	272.7 ± 46.3	
Tissue samples ^a			
Tumour	87	289.2 ± 29.6	0.0005 ^b
Non tumour	230	74.5 ± 8.3	
Lymph node ^a			
Positive	28	296.3 ± 49.2	0.40
Negative	59	283.6 ± 37.0	
Stage (TNM) ^a			
I	7	148.5 ± 16.8	* 0.0005 ^b ** 0.01 ^b
II	52	306.5 ± 40.9*	
III	23	277.5 ± 48.5**	
IV	5	488.7 ± 116.2**	
NT distribution ^a			
PE	69	61.2 ± 8.4	*** 0.025 ^b
N	87	91.5 ± 12.9 ***	
DE	74	67.2 ± 9.8	

Mean ± SEM (indicate standard error of the mean).

^a Clinical associations and *P* values were analysed by Student's *t* test

^b *P* < 0.05 was considered statistically significant.

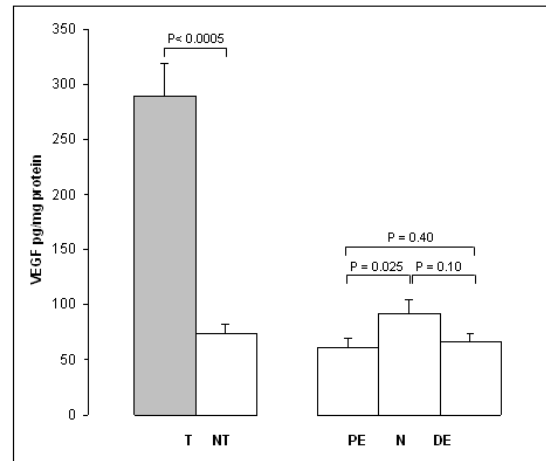


Figure 1

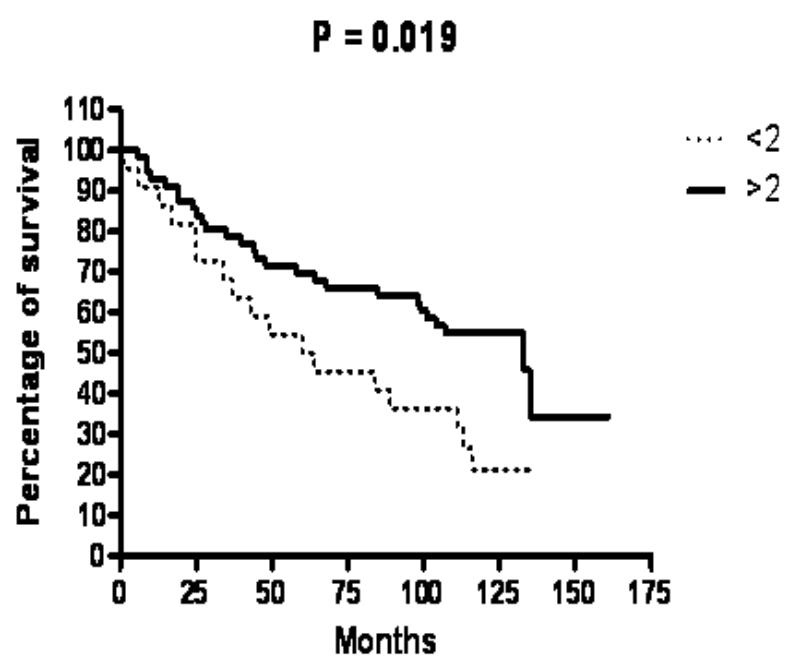


Figure 2